

From the Department of Histology, University of Lund,
Lund, Sweden

NEUROGENIC MECHANISMS IN THE CEREBRO - VASCULAR BED

AUTONOMIC NERVES, AMINE RECEPTORS AND THEIR EFFECTS
ON CEREBRAL BLOOD FLOW

BY

LARS EDVINSSON

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BY

LOUISIANA



June 1972

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The present dissertation is based on the following ten papers:

1. EDVINSSON, L., K.C. NIELSEN, CH. OWMAN and B. SPORRONG, Cholinergic mechanisms in pial vessels. Histochemistry, electron microscopy and pharmacology. Z. Zellforsch. 1972. 134. 311-325.
2. EDVINSSON, L., CH. OWMAN, E. ROSENGREN and K.A. WEST, Concentration of noradrenaline in pial vessels, choroid plexus, and iris during two weeks after sympathetic ganglionectomy or decentralization. Acta physiol. scand. 1972. 85. 201-206.
3. EDVINSSON, L., M. LINDVALL, K.C. NIELSEN and CH. OWMAN, Are brain vessels innervated also by central (non-sympathetic) adrenergic neurones? Brain Res. 1973. 63. 496-499.
4. EDVINSSON, L., K.C. NIELSEN and CH. OWMAN, Influence of initial tension and changes in sensitivity during amine-induced contractions of pial arteries in vitro. Arch. int. Pharmacodyn. 1974. 208. 235-242.
5. EDVINSSON, L., and CH. OWMAN, Pharmacological characterization of adrenergic alpha and beta receptors mediating vasomotor response of cerebral arteries in vitro. Circ. Res. 1974. 35. 835-849.
6. EDVINSSON, L., B. FALCK, and CH. OWMAN, Possibilities for cholinergic nerve action on smooth musculature and sympathetic axons in brain vessels mediated by muscarinic and nicotinic receptors. Circ. Res. 1975. (in press).
7. EDVINSSON, L., and CH. OWMAN, A pharmacologic comparison of histamine receptors in isolated extracranial and intracranial arteries in vitro. Neurol. 1975. 25. 271-276.
8. EDVINSSON, L., P. AUBINEAU, CH. OWMAN, R. SERCOMBE and J. SEYLAZ, Sympathetic innervation of cerebral arteries: Prejunctional supersensitivity to norepinephrine after sympathectomy or cocaine treatment. Stroke 1975. (in press).
9. EDVINSSON, L., CH. OWMAN and N.-O. SJÖBERG, Autonomic nerves, mast cells, and amine receptors in human brain vessels. A histochemical and pharmacological study. Brain Res. 1975. (in press).

10. SERCOMBE, R., P. AUBINEAU, L. EDVINSSON, H. MAMO, CH. OWMAN, E. PINARD and J. SEYLAZ, Neurogenic influence on local cerebral blood flow. Effect of catecholamines or sympathetic stimulation as correlated with the sympathetic innervation. Neurology 1975. (in press).

In the text they will be referred to by their Arabic numerals.

INTRODUCTION

The cerebral blood circulation is generally believed to be controlled by chemical factors. Thus, the arterial blood gas tensions, $p\text{CO}_2$ and $p\text{O}_2$, cause alterations in the perivascular pH which ultimately trigger changes in the blood flow (11-14).

The possibility of a neurogenic influence on the cerebral blood vessels has for a long time been disputed, but its physiological role has been regarded as negligible. The use of modern histochemical methods which have revealed, beyond doubt, that the intracranial vascular bed receives a well-developed adrenergic (15) and cholinergic (1) nerve supply has formed the basis for intensified studies on neurogenic mechanisms. Results obtained during the last few years have offered strong support for the view that autonomic nerves can, indeed, influence cerebral blood flow. It is to be expected that, in the near future, it will be possible to define the more precise role of such neurogenic mechanisms in the physiological control of the cerebral circulation.

Experience from advanced physiological studies on such systems as the coronary, mesenteric, or renal vascular beds has clearly shown the necessity for a detailed knowledge about several basic anatomical and pharmacological neuro-receptor mechanisms for an adequate interpretation of phenomena shown in physiological studies in animals as well as humans. Mechanisms including the identity, distribution, and origin of the perivascular nerve fibres, the relationship of such fibres to the vascular smooth musculature, the characteristics of the receptor apparatus mediating the vasomotor effect of vaso-active amines — e.g. the autonomic transmitters — and the possible interaction between the systems of perivascular autonomic nerves. Such anatomical and pharmacological prerequisites have been investigated in the present series of studies. Some of the results obtained have been used to design a preliminary series of experiments to elucidate the changes in local cerebral blood flow induced by nerve stimulation and exogenous vasoactive amines.

VASCULAR MORPHOLOGY

The blood is carried to the brain via two routes: the common carotid and the vertebral arteries. The common carotid artery gives off the internal carotid artery and the external carotid artery. The latter splits up in several branches to supply the extracranial tissue with blood (16). The internal carotid artery is vestigial in some mammals, such as the adult cat (17). Under these conditions the brain is supplied with blood via the ascending pharyngeal, the vertebral, the external carotid by way of the internal maxillary artery and the rete externum (carotid plexus) (14, 17).

The vertebral arteries, which are derived from the subclavian arteries, join on the ventral side of the spinal cord to form the basilar artery. This artery gives off branches (aa. cerebellaris and pontine branches) and finally join the posterior part of the circle of Willis. This network of communicating arteries is important for interchange of blood on the ventral side of the brain in the region of the hypophysis. The blood is further distributed from these arteries to the other extracerebral (or pial) bed, which forms a network on the outer side of the brain, and then continues to the vessels of the intracerebral bed (in the brain parenchyma).

Extracerebral (pial) vessels

The histological appearance of the pial vessels can be summarized in the following way (18-21): The adventitial layer is notably thin. It is composed of a layer of loose connective tissue intermingled by a dense plexus of adrenergic and cholinergic nerves. These nerves are grouped together within the same Schwann cell sheath, and in their terminal course they have a close relation to the outermost layer of the vascular smooth muscle cells. The tunica media consists of a population of smooth muscle cells. The brain vessels are of the muscular type, but occasional elastic sheets may be found between successive layers of muscle cells. Myofilaments can be seen in the muscle cell cytoplasm, as well as dense bodies underlying the cell surface, which are thought to be "attachment bodies" anchoring the myofilaments to the cell surface. Small

arterioles have only a single layer of smooth muscle cells. Each of these muscle cells are surrounded by an envelope of basement membrane, which may be shared with adjacent cells or with the underlying endothelial sheet. Myofilaments that have a straight course are seen to terminate in a dense "attachment device" which seems to anchor them to the cell surface. Sometimes muscle cells can be seen to share a single basement membrane or the space between them is occupied by an incomplete basement membrane. This arrangement offers the possibility for excitation spread of impulses emanating from the vasomotor nerves lying in the adventitial border. The endothelial sheet sometimes rests upon an internal elastic membrane; otherwise the endothelial cells form the only layer of the tunica intima.

Intracerebral (parenchymal) vessels

The arteries penetrating the cerebral cortex arise from the distributing pial vessels lying in the subarachnoid space. They have a short extracerebral course before entering the brain parenchyma. Their size is successively diminished and they are mainly of three types: arterioles, capillaries and venules (22-24). The arterioles are characterized by at least one layer of smooth muscle cells in their wall. Sometimes a single muscle cell may cover the entire vessel circumference. The endothelium consists of a continuous layer of endothelial cells which lack fenestrations. The endothelial cells are joined together by tight junctions which prevent large molecules to pass freely into the brain tissue proper. Another special feature is the relatively low number of pinocytotic vesicles (caveole) in the endothelium. The thin adventitial layer is separated from the neuropil by an adventitial space (which is not a true, circumferential perivascular space) and a distinct basement membrane, which is continuous with the basement membrane covering the surface of the brain. This adventitial space may contain macrophages, fibroblasts, Schwann cells, and autonomic nerve fibres. The capillaries and venules are often indistinguishable from each other. They have a continuous endothelium and sometimes pericytes, and all is surrounded by a basement membrane. Capillaries without pericytes have the basement membrane as the only structure separating the endothelium from the surrounding cortical parenchyma.

INNERVATION OF BRAIN VESSELS

Already in the early studies by Willis in 1664 (16) nerve fibres were described on the anterior and posterior cerebral arteries. These findings were confirmed at the end of the nineteenth century by Benedikt (25) and Aronson (26), who also noted that part of these fibres originated in the sympathetic plexuses around the major vessels at the base of the brain. This finding of a sympathetic origin of many of the perivascular nerves was somewhat later verified by Bochenik (27) and Hüber (28). Since then, many reports have described the distribution of these nerves (29-33) by the light microscope using silver impregnation techniques.

Adrenergic innervation of pial vessels (1, 2)

Not until the introduction of modern histochemical techniques (see 34, 35) was it possible to define the origin, distribution and identity of the nerves to the cerebral blood vessels. A well-developed plexus of sympathetic adrenergic nerves has been visualized in the pial vessels of a variety of laboratory animals: mouse (1), rat (1, 36-41), hamster (1), guinea-pig (1, 37, 38), rabbit (1, 10, 42, 43), cat (1, 15, 36-38, 44), dog (45, 46), and monkey (47, 48). The histo-fluorescence technique has shown that the construction of the sympathetic innervation in the pial system closely resembles that found in other parts of the circulation.

The following technique was applied for studies of the adrenergic nerves to the pial vessels in the present investigation: Pieces of vessels were dissected out, cut open longitudinally and stretched flat on microscope slides, followed by drying in a desiccator over phosphorous pentoxide in vacuo for 1 hour. Subsequently, the tissue preparations were exposed to formaldehyde gas at 80°C for 1 hour (15). In some cases, tissue pieces were frozen in a propane-propylene mixture to the temperature of liquid nitrogen. This was followed by freeze-drying and formaldehyde treatment. Such specimens were cut on a microtome at a 6 μ thickness and mounted in Entellan before the microscopy (34). In some instances the glyoxylic acid technique was utilized in conjunction with Vibratome sectioning (see p. 12).

Fluorescence microscopy revealed an adrenergic nerve plexus located in the adventitia at the outer border of the media layer. The arteries usually received a richer supply than the veins. This pattern could be followed in arterioles with diameters down to $15\ \mu$. Small vessels were sometimes accompanied only by one nerve fibre. Since few muscle cells were present in the wall, this innervation should be satisfactory for the functional demands. The single nerve fibres were characterized by the presence of typical, intensely fluorescent enlargements on the nerve terminals, the varicosities. Extensive studies (see 49) have shown the transmitter to be stored in special granules (or vesicles) within the adrenergic neurons. They occur in all parts of the sympathetic adrenergic neuron, including the cell bodies, the non-terminal axons and the terminals, although only the terminals contain high concentrations of noradrenaline. The reason for this widespread distribution of the amine is that the transmitter granules are formed in the cell bodies and transported down the axon (by axoplasmatic flow), to pile up in the varicosities of the terminals. Noradrenaline is released from the terminals into the synaptic cleft to act on specific receptor sites.

The sympathetic adrenergic nerves of the pial vessels originate in the superior cervical ganglia as revealed by denervation experiments showing the total disappearance of specific fluorescence (1,8,10,15,39) and of chemically measured noradrenaline (2) within 3 days postoperatively. The innervation was found to be strictly unilateral. The ascending nerves accompanied the internal carotid artery and had a characteristic distribution in the vessels belonging to the circle of Willis. The caudal part of the vertebral arteries and the spinal cord arteries were supplied with nerves from sympathetic ganglia other than the superior cervical since some of the noradrenaline-containing nerve fibres persisted after excision of the superior cervical ganglia.

The anterior and middle cerebral, internal carotid and posterior communicating arteries had a denser network of adrenergic nerves than that found in the posterior cerebral, cerebellar, basilar and vertebral arteries. This was calculated by counting the number of nerve fibres per diameter of the vessel. As can be seen in Table I the carotid part has roughly 40-50 fibres while the

TABLE I

NUMBER OF ADRENERGIC (FORMALDEHYDE HISTOCHEMISTRY) AND
CHOLINERGIC (CHOLINESTERASE HISTOCHEMISTRY) NERVES IN
VARIOUS PIAL ARTERIES FROM CATS

Vessel	Adrenergic nerves	Cholinergic nerves
Anterior cerebral artery	36 ± 3	36 ± 2
Middle cerebral artery	41 ± 4	41 ± 3
Posterior cerebral artery	19 ± 2	25 ± 2
Internal carotid artery	52 ± 6	47 ± 5
Cerebellar arteries	12 ± 1	13 ± 2
Basilar artery	22 ± 2	22 ± 2
Vertebral artery	18 ± 3	24 ± 2

The values are expressed as mean $\pm$ S.E.M. of "meshes" per vessel diameter as counted in spread-preparations of 5 arteries of each kind. Each "mesh" in the autonomic nerve plexuses surrounding the various vessels consisted of one or a couple of histochemically visible fibres. Larger bundles of fibres present in, for example, the basilar artery were not included in the estimation.

posterior part of the circle of Willis, basilar and vertebral vessels have (12-22) fibres revealing a heterogeneity in the pattern of innervation. This difference could be due to the fact that the phylogenetically older regions (the posterior part of the brain and the brain stem) are less well influenced by sympathetic mechanisms than younger parts of the brain (cortical structure).

Pial vessels, choroid plexuses and irides are three structures which are innervated by adrenergic nerves from the superior cervical ganglia. They have been compared quantitatively (2) by measuring noradrenaline fluorometrically (50, 51). The changes that occurred after denervation and decentralization of the effector tissue during 2 weeks were studied. The noradrenaline levels fell to undetectable levels at about 2 days after ganglionectomy in all three organs and remained low during the 2-week period studied. This was confirmed by fluorescence microscopy and it was, furthermore, shown that no reinnervation had taken place even at 6 months postoperatively (8). The fall was somewhat slower in the choroid plexus. After the ganglionectomy there is a leakage of noradrenaline from the nerves which caused an activation of the receptors during the first phase of nerve degeneration (52, 53). Previous studies have stated that preganglionic sympathetic denervation does not alter the organ content of noradrenaline (54). However, the noradrenaline level increased in the pial vasculature after decentralization (by 40 per cent). In the iris and choroid plexus the noradrenaline levels were initially slightly reduced. The level was later normalized.

Adrenergic innervation of intracerebral vessels (3)

The adrenergic nerve plexuses enclosing the pial arteries also accompanied several of the arterial branches issuing into the brain parenchyma (3, 10). The arteries have a small calibre and the network of axons is fairly sparse. Also many of the intracerebral arterioles are accompanied by one or a couple of varicose fluorescent fibres. All these nerve fibres, like those supplying the pial circulation, disappeared after bilateral removal of the superior cervical ganglia (10). It should be pointed out that the density of innervation of the vessels is not necessarily related only to the number of nerve terminals present

but obviously also to the amount of smooth musculature supplied by the nerves. The intracerebral arteries and arterioles are very thin-walled, the media being composed only of a single layer of smooth muscle cells. In a microscope cross-section, the lumen can sometimes be seen to be incircled by only one muscle cell. The small number of fluorescent nerves and small volume of smooth musculature may thus represent an adrenergic innervation of intracerebral arterioles as rich as that seen in other parts of the circulatory system. Recent electron-microscopic data (24) have shown that also the intracerebral — like the pial — arterial system is innervated in a true structural sense. There is fluorescence microscopic evidence that the amount of sympathetic nerves to intracerebral vessels is related to the density of adrenergic innervation of the pial artery supplying each region. For example, in the caudate nucleus which receives blood from the richly innervated middle cerebral artery, half of the arterioles have sympathetic nerves whereas in the lateral geniculate body, supplied from the poorly innervated posterior cerebral artery, only few of the arterioles are accompanied by sympathetic fibres (10).

A close association of intracerebral adrenergic nerve fibres to the wall of the arterioles in the brain parenchyma has been shown with the formaldehyde, glyoxylic acid, and immunohistochemical techniques (3, 10, 41, 55–57). It has, further, been postulated that these fibres originate in the locus coeruleus (55, 58). To test this, bilateral electrolytic lesions were placed in locus coeruleus using a Kopf stereotaxic instrument (57). Two weeks later the animals were killed and various brain regions studied with the glyoxylic acid histofluorescence method following Vibratome sectioning (59). The lesion had destroyed all cell bodies within locus coeruleus. Although a marked reduction in non-sympathetic perivascular adrenergic nerves was produced, a considerable number remained — particularly in the hypothalamus — in accordance with the fact that cerebral noradrenaline nerves originate from several other nuclei in the brain stem besides locus coeruleus (60). Therefore, more extensive lesions were made using 6-hydroxydopamine which selectively destroys the catecholamine fibres. Thus, all ascending catecholamine nerves were lesioned by bilateral stereotaxic injection of 8 μ g 6-hydroxydopamine in 4 μ l saline (60) into the central tegmental tract in the caudal mesencephalon, and various brain regions were studied

by the glyoxylic acid method at least 2 weeks after the injection. This treatment resulted in a complete or almost complete disappearance of fluorescent catecholamine nerves in the brain and, hence, also in the vicinity of cerebral arterioles. Thus, the nerves in question originate in the brain stem, but only partially from locus coeruleus (57).

A series of electronmicroscopic studies was performed on cats to elucidate whether the perivascular catecholamine nerves of intracerebral origin really innervate the smooth musculature of brain arterioles (57). Axons, one or several around the circumference of the vessel close to its outermost smooth muscle layer, were found in 66 % of the arterioles in unoperated cats. At 24 hours after sympathectomy, several perivascular axons showed degenerative signs, whereas at later stages no axons could be found in any of the arterioles. This (a) shows that intracerebral arterioles are devoid of non-sympathetic (i.e. cholinergic) nerves, and (b) shows that the sympathetic fibres are the only adrenergic system innervating intracerebral arterioles in a conventional manner. In some instances it could be seen how the cerebral neuropil, containing axons with electron-dense vesicles, extended very close to arteriolar walls, though always with a thick intervening basement membrane, which might well correspond with the close vascular association of parenchymal nerves observed at the level of optical microscopy. If such axons functionally influence the cerebral microcirculation, we obviously have to accept an unconventional kind of neuro-vascular relation, in which very long diffusion distances for the adrenergic transmitter substance is involved. The hypothesis of a central noradrenergic regulation of brain microcirculation was recently tested in functional experiments (61). Local injection of carbacholine into the locus coeruleus area produced a reduction in the cerebral blood flow and an increase in the blood-brain barrier permeability. The intraventricular administration of the alpha blocker phentolamine had the opposite effects. The relevance of these experiments is at present difficult to assess: the experimental interference involves a very complex area in the brain stem where possibilities for effects on neighboring structures must be very difficult to avoid, the technique (62) used for the flow measurements — clearance of radioactive water (H_2^{15}O) — is unusual

and therefore its precision and reproducibility are not sufficiently well established (particularly under conditions involving changes in barrier functions and hence diffusion processes) and, moreover, only a very limited number of animals (4 monkeys) has been used in the fairly traumatizing experiments.

Cholinergic innervation of pial vessels (1)

The histochemical cholinesterase method, in combination with appropriate enzyme inhibitors, is at present the only reliable method for demonstration of cholinergic nerves at the level of optical microscopy (35, 63). It has presently been used to localize and study the distribution of acetylcholinesterase-containing nerves. Vascular segments were, after removal from the cat's brain, mounted flat on microscope slides and dried in vacuo. After optimal incubation conditions, and after preincubation with Mipafox to inhibit pseudocholinesterase, the acetylcholine activity was indicated by small brownish granules which formed a plexus outlining nerve fibres.

The extent and distribution of the cholinergic nerves were the same as the arrangement of the adrenergic nerves (Table I). Thus, a greater number of nerves per vessel diameter was constantly observed in the anterior than in the posterior part of the pial circulation. The plexus of cholinergic nerve fibres was demonstrated in vessels with diameters down to $15\ \mu$, located both at the base of the brain and on the convexities. The veins were less well supplied with cholinergic nerves than the arteries. This pattern of innervation was essentially the same in all the laboratory animals studied (mouse, rat, rabbit, hamster, guinea-pig, and cat) and in preliminary experiments also confirmed in dog, cow, pig, and monkey. The findings are in line with those previously made on cat (64-67) and dog (46) by other investigators. In accordance with the electronmicroscopic findings that all perivascular nerves in brain parenchyma disappeared after sympathectomy (57), no cholinesterase-positive fibres were found around intracerebral vessels.

The origin of the cholinergic nerves is still unknown. It has been claimed in the older literature that the parasympathetic nerves to the intracranial vessels run in the facial nerve, turn off in the region of the geniculate

ganglion, to continue in the greater superficial petrosal nerve to the plexus of the internal carotid artery where they form synapses (68, 69). When the intracranial parts of the cranial nerves were cut no loss of acetylcholinesterase in the pial cholinergic nerves was observed within 2 weeks (unpublished observations). There is thus reason to believe that cholinergic ganglion formations may be located very close to the effector system. In fact, cholinergic ganglion cell formations have been identified in the adventitia of the intracranial part of the internal carotid artery and in the carotid sinus (46). Excision of the superior cervical ganglia did not overtly affect the number and distribution of the cholinergic nerves.

The regional distribution of the cholinesterase-containing nerve plexuses closely resembled those of the adrenergic. This fact gave us reason to analyze the arrangement in some more detail (1). At first, the whole-mounts of pial vessels were exposed to mild formaldehyde gas treatment (for demonstration of adrenergic nerves) and, after photography, this was followed by extended incubation in acetylthiocholine (to show cholinergic nerves). Analysis showed that the two systems of fibres ran side-by-side in the strands of the autonomic nerve plexuses.

AMINE RECEPTORS IN BRAIN VESSELS

Pial vessels as an in vitro model (4)

In several vascular beds, a large number of investigations have been performed with simple in vitro systems for registration of vasomotor functions to establish the receptor mechanisms involved in the vascular response. It is therefore surprising that the receptor mechanisms in such a complex and controversial system as the cerebral circulation has not previously been examined. This might be due to an early failure to obtain contractile response in cerebral resistance vessels (70). Reproducible results were not achieved until 10 years later when a sensitive method was devised utilizing a rigid system in which a whole vascular cylinder could be tested (71). A variety of drugs has now been studied for their vasomotor action in cerebral vessels (71-84). However, these studies

either do not contain sufficient quantitative information or they have not taken into consideration certain experimental precautions necessary for a detailed characterization of the receptor mechanisms (see ref. 85).

We have used the middle cerebral artery of cats as model in the receptor analysis (4-8) because it is well innervated by adrenergic (15) and cholinergic (1) nerves and its size (5) is suitable for recordings of mechanical activity. This artery has turned out to be a representative model for the brain vasculature in the sense that very similar results have been obtained on isolated preparations from pial arteries of the brain convexities in humans (9) and from intracerebral small arteries in baboons (unpublished observations). As an initial methodological study the recording system was tested with regard to the influence on the contractile response caused by variations in the initial tension of the vessel (4). The amount of contraction increased with increasing passive tension up to 500 dyn and then successively decreased with further stretching of the vessel. An initial tension of 400 dyn was therefore chosen for practical purpose. The tension in the pial vessel wall in vitro was found to be 800 dyn/mm² (5), which is less than the corresponding value calculated for the in vivo situation. This means that the preparation was not overstretched during the experiments.

The dose-response relations of noradrenaline, phenylephrine, isoprenaline, and acetylcholine were tested hourly to elucidate any time-dependent changes. The results showed that the ED₅₀-values exhibited little variation in the various test runs. However, the E_{Am} levels remained unaltered only during a fairly limited period, shown to be 2-5 hours after mounting of the preparation. Thus, when applying an initial passive tension of 400 dyn and when the tests are carried out within the above-mentioned time-period, the model can be used for reproducible characterization of amine-induced vasomotor actions.

Our knowledge about the mechanisms for the interaction between drugs and receptors as an operational approach to characterize various amine receptor mechanisms is to a large extent based on the fundamental studies by Furchgott; several excellent reviews are available, the one from 1972 being particularly comprehensive (85). A drug-receptor system is composed of the tissue

component that interacts with the agonist (pharmacologic receptor) and the complete sequence of events that connects the receptor with the response mechanism. As is seen below, the pial arteries contain a variety of pharmacologic receptors. The way to identify the presence of these sites is either by obtaining responses to various agonists and compare the potencies or show that the response can be antagonized by specific receptor blocking agents. As long as the true receptors have not yet been isolated, we have to depend on the assumption that similarities or differences in their response also reflect similarities or differences in the characteristics of the receptors mediating these responses. In this way numerous examples of receptor types have been discovered in smooth muscle tissue. Schild (86) has demonstrated that responses to agonists interacting with the same receptor are blocked to a similar degree by a given competitive antagonist. From this follows that the action of an agonist on various tissues will be antagonized to the same extent by a competitive blocking agent if the receptors for the agonist in those tissues are identical. He introduced the concept of the pA_2 (log concentration of the antagonist necessary to increase the ED_{50} by a factor of 2) to quantify this relationship. A similar measure of antagonism is represented by the dissociation constant (K_B) of the receptor-antagonist complex which is the relation between the concentration of antagonist and dose ratio minus 1 (87). Another quantitative means to define the receptors is by the dissociation constant (K_A) for the receptor-agonist interaction (85). This requires the use of an irreversible inhibitor (e.g. dibenamine or phenoxybenzamine) which can block a fraction of the receptors. The doses producing equal responses before and after this reaction can be used to calculate K_A . Further, at equilibrium there exists a relation between the fraction of receptors occupied and the response which makes it possible to achieve information of this relation.

Using the previously mentioned technique, care being taken to fulfill accepted precautions necessary to allow for quantitative characterization, the following amine receptors were studied:

Alpha-adrenergic receptor (5)

This receptor was studied on resting vessels. Adrenaline, noradrenaline, isoprenaline, and phenylephrine contracted the pial artery in a dose-dependent way and in the mentioned order of potency. It was notable that they had relatively high ED_{50} -values as compared to other vascular systems. The order and degree of relative potency found for the amines mediating contraction were in accord with those reported for alpha responses in other tissues (85), with the exception of the high ED_{50} and low E_{Am} for phenylephrine. The bracketing procedure showed that this agent acted as a partial agonist in the feline middle cerebral artery. In fact, this finding was recently confirmed by Kuschinsky (personal communication) in microapplication studies on cat's pial vessels in situ. The reversible alpha-receptor antagonists, piperoxan and phentolamine, in increasing concentrations caused a parallel shift in the dose-response curves obtained with adrenaline, noradrenaline and phenylephrine. The dissociation constant (K_B) of piperoxan was found to be 2.92×10^{-7} M with phenylephrine as agonist, and 1.24×10^{-7} M with noradrenaline. The inhibitor alone did not overtly affect the tone of the arteries in the doses usually employed in these inhibition experiments, whereas it produced a contraction of about the same magnitude as the catecholamines (about 250 dyn) at high concentrations. This shows that the inhibitor has good affinity and low intrinsic activity in the doses used.

The irreversible alpha-antagonists, phenoxybenzamine and dibenamine, inhibited the contractions of adrenaline, noradrenaline and phenylephrine. The antagonism usually consisted of a parallel shift combined with a decrease in the E_{Am} . The dissociation constant for the receptor-agonist complex (K_A) was calculated for noradrenaline (tested on sympathectomized vessels), using the dose-response curves before and after partial irreversible blockade of the alpha-receptor with phenoxybenzamine. A plot of reciprocals of equiactive concentrations of noradrenaline before and after partial blockade resulted in a straight line. The slope and intercept of the line were used to calculate the K_A -value, which in this case was found to be 1.73×10^{-6} M. The K_A -value for noradrenaline on the alpha-receptor for cat's pial artery was equal to those

obtained from rat's portal vein (88) and rat's aorta (Furchgott, personal communication), but in variance with rabbit's aorta which had a 10 times lower value (89). The fraction of receptors used to produce ED_{50} was almost equal in all these tissues (90). Comparison of relative potencies of nine alpha-agonists has offered further indications for a variability in the characteristics of alpha-receptors in different vessels (91). The observations made in our experiments also showed that (a) the noradrenaline response was not directly proportional to the fraction of receptors occupied, (b) half maximum contraction to occur with only 11 per cent receptor occupation and (c) maximum response was achieved when 75 per cent of the receptors are occupied by the adrenergic transmitter. These facts, together with the presently obtained K_B -values, high ED_{50} -values and the finding that the specific alpha-stimulant (phenylephrine) acted as partial agonist, strongly suggest the presence of an unusual type of alpha-receptor in brain vessels.

Beta-adrenergic receptor (5)

The beta-receptor function was tested on vessels which were given an active tone by 5×10^{-6} M serotonin. Isoprenaline, adrenaline, noradrenaline and terbutaline all dilated the tonically contracted artery in a dose-dependent manner, the relative potencies being isoprenaline > noradrenaline = adrenaline > terbutaline. The inhibition of the dilation produced by isoprenaline or terbutaline by propranolol was competitive, as confirmed by Arunlakshana-Schild plots (92). The dissociation constants for propranolol with terbutaline (2.31×10^{-9} M) or isoprenaline (1.77×10^{-9} M) as agonists were indistinguishable. The dilatory action of isoprenaline was in another series also found to be inhibited in a competitive manner by the selective beta₁-receptor antagonist practolol given in low concentrations (unpublished observations). These results, together with the finding that noradrenaline was almost equipotent to adrenaline and that isoprenaline was about 2,500 times more potent than terbutaline, strongly indicated that the beta-receptor was of the beta₁ type. This receptor type has also been found in the coronary arteries, whereas other parts of the peripheral circulatory bed possess beta₂-receptors (85, 93).

Cholinergic receptors (6)

Histochemistry and electron microscopy have revealed the presence of a cholinergic nerve supply in the pial vessels which is as well-developed as the adrenergic innervation (1). There is some evidence in the literature that the intracranial arteries are supplied also with afferent nerve fibres. They are considered to pass via the vertebral plexus along the posterior cerebral, basilar, cerebellar, and vertebral arteries into the trigeminal, glossopharyngeal and possibly also vagus nerves (see 20, 94). The amount of acetylcholinesterase in afferent (sensory) nerves appears to be so low that a substantial staining is obtained only after prolonged incubation (95). This would mean that acetylcholinesterase staining under usual conditions, such as those employed in the present study, only reveals the efferent (parasympathetic) fibres. It is a general experience from histochemical studies on numerous peripheral tissues in our laboratory that other vascular beds are devoid of cholinergic nerve supply. There is reason to believe that the positive cholinesterase staining in perivascular nerves reported by El Badawi and Schenk (96) may reflect the presence of pseudocholinesterase in sympathetic fibres since appropriate inhibition of this enzyme did not appear to have been employed. In such peripheral vessels as the aorta (97), ear artery (98), and portal vein (98a) acetylcholine has been found to induce a vasomotor action in the vessels in a dose-dependent manner, and the response was inhibited in a competitive manner with atropine. Acetylcholine showed a dual effect on isolated pial vessels, involving both dilation and contraction. In order to ascertain that the effect was direct on the smooth musculature, and not mediated by adrenergic nerves, the vessels had been sympathectomized beforehand. In resting vessels the dilatory action was weak whereas it could be enhanced in tonically constricted vessels. The dilatory response occurred at concentrations between 10^{-8} – 10^{-6} M whereas the contractile action started at about 3×10^{-6} M of acetylcholine in the bath. Both effects were inhibited by atropine in a competitive manner as confirmed by Arunlakshana-Schild plots. The dissociation constant for the drug-receptor complex was 3.85×10^{-11} M for the dilation (analysed in a tonically constricted vessel) and 1.12×10^{-11} M

for the contractile effect (previously relaxed vessels). The antagonism by atropine, and the fact that hexamethonium did not affect the acetylcholine response, indicated that the two effects were mediated by cholinergic receptors of the muscarinic type. It is conceivable that the cholinergic vascular receptors mediate vasodilation during physiological conditions whereas high, pharmacological, doses of parasympathomimetics produce vasoconstriction.

The adrenergic and cholinergic nerve terminals lie close together within the same Schwann cell sheath, separated only by a 25 nm distance (1). This arrangement opens the possibility for an interaction process between the two neuron systems at the axo-axonal level. Previous studies on peripheral tissues (lacking cholinergic nerves) have shown that the adrenergic fibres possess muscarinic inhibitory receptors. This was concluded on the basis of experiments showing that a variety of muscarinic agonists selectively inhibited the vasoconstriction evoked by peri-arterial nerve stimulation, which was abolished by atropine (see 99). In order to elucidate the possible interaction process between the two neuron systems, the noradrenaline release evoked by transmural stimulation of pial arteries under the influence of parasympathomimetic compounds was studied in an efflux system (100). Pial vessels were pre-incubated in a Krebs-Ringer buffer containing 10^{-7} M of ^3H -noradrenaline for 20 min. The vessels were then placed in a small chamber where the preparations were superfused continuously by the same buffer and the superfusate collected for measurement of tritium activity by liquid scintillation (6).

The general aspects of the superfusion model and its validity for studies on adrenergic release processes have been discussed in detail by Farnebo (100). The reliability of the model in the present experiments was checked by sympathectomy or by administration of the adrenergic neuron blocking agents, guanethidine or bretylium.

Electrical field stimulation with 10 Hz at a pulse duration of 1 msec increased the tritium efflux by approximately 125 per cent, the effect being further enhanced by the presence of hexamethonium. On the other hand, both acetylcholine and nicotine inhibited the effect of stimulation so that the tritium efflux was only insignificantly higher than that occurring spontaneously in the

absence of stimulation. This inhibitory action of the parasympathomimetics was counteracted by hexamethonium but not by atropine. These pharmacological experiments show the existence of an interaction between the two types of autonomic nerve terminals — the cholinergic ones being capable of inhibiting the electrically induced overflow or release of tritium from the adrenergic terminal by means of the nicotinic type of cholinergic receptors.

Acetylcholine, liberated from the cholinergic nerves may therefore, under physiological conditions, induce pial vasodilation by a combination of (a) direct effects on muscarinic receptors in the vascular smooth muscle and (b) through axo-axonal interaction with the nicotinic receptors in the adrenergic nerves causing inhibition of noradrenaline release, hence allowing for a concomitant reduction in sympathetic vascular tone.

Histamine receptors (7)

There is much experimental evidence suggesting that histamine in brain is stored in intracerebral neurons (101). Histochemical and chemical studies have shown that histamine is also present in cerebral mast cells, often with a perivascular localization (102). This opens an obvious possibility for a local action of histamine on brain vessels and, for this reason, experiments were done to characterize histamine receptors using the same model as that previously applied for the autonomic receptors. With regard to the proposed role of histamine in the pathophysiology of migraine the studies also included a comparison of histamine responses in intracranial and extracranial arteries (*i.e.* branches from the maxillary and lingual arteries).

The contractile response was studied in the presence of 10^{-4} M burimamide to avoid interference with dilatory mechanisms. In the extracranial arteries histamine produced a strong contraction, approximately 850 dyn, whereas the contractile response of the intracranial arteries was only about one third and the ED_{50} concentration was high. This and the finding that the anti-histaminics, chlorpheniramine and mepyramine, in increasing doses reduced slope and maximum response of histamine in the middle cerebral artery indicated that the contraction was not mediated by specific histamine receptors. In the extracranial arteries, on the other hand, these antagonists produced a clear parallel shift of the histamine-induced contraction. Experiments with mepyramine

revealed competitive antagonism, demonstrating the presence of histamine H_1 -receptors with a mean K_B -value of $3.96 \times 10^{-9} M$.

The dilatory response was analyzed after the arteries had been given a tonic contraction with serotonin to magnify the response. The bath also contained $10^{-4} M$ mepyramine to block the above-mentioned contractile effect of histamine. Under these conditions, histamine caused a dilation with a low ED_{50} in both types of vessels. A parallel shift in the dose-response curves was obtained with graded doses of burimamide, demonstrating that the dilation was mediated by histamine H_2 -receptors. The K_B -values were $2.03 \times 10^{-6} M$ for the intracranial and $1.78 \times 10^{-6} M$ for the extracranial arteries.

Based on these findings, it was concluded that the specific vasomotor action of histamine in the brain involves only dilatory histamine H_2 -receptors whereas the contractile effect of histamine is non-specific. This is in contrast to extracranial arteries where the presence of both contractile (H_1) and dilatory (H_2) histamine receptors could be established.

THE NEURO-MUSCULAR RELATION

Ultrastructural aspects

Pease and Molinari (18) were the first who, in electronmicroscopic studies, showed the presence of nerves in the pial vessels. The fibres ran in the adventitial layer, and were always associated with Schwann cells. The absence of nerves or nerve endings in the medial layer was thought to imply that these vessels did not receive any functionally important innervation. Since then, it has been widely observed in various vascular beds (see 103) that nerves with a documented vasomotor function usually do not penetrate in among the smooth muscle cells in the media. The portal vein of the rat (98 a) and wing veins of bats (104) are exceptions from this pattern. The observations on the pial vessels have since been confirmed in several studies (19, 22, 103, 105, 106) and it was further observed that the nerve endings contain both granular and agranular synaptic vesicles, indicating the presence of adrenergic and non-adrenergic axons. The shortest distance by which the axon terminals approach

the surface of the outermost smooth muscle cells in the media was 80 nm (1, 19, 20, 103, 106, 107). The further identification of the perivascular nerve fibres was studied by (a) sympathectomy, which eliminated all terminals with small (50 nm) granular vesicles concomitant with the disappearance of all the fluorescent nerves, (b) injection of the false adrenergic transmitters, 6-hydroxydopamine or 5-hydroxydopamine (to the non-denervated animal), after which vesicles of adrenergic axons could be distinguished by the presence of highly electron-dense cores in the synaptic vesicles, and (c) permanganate fixation, by which the catecholamine content in the synaptic vesicles could be selectively visualized (1, 39, 40, 107). Taken together, the studies show that both adrenergic and non-adrenergic fibres, present in approximately equal number, supply the pial arterial system in a manner which is fully accepted as a functioning vascular innervation. On the basis of the cholinesterase histochemistry at the light-microscopic level it is conceivable that the non-adrenergic nerves are, in fact, cholinergic. In autonomically innervated structures such as the heart and iris where both adrenergic and cholinergic nerves are present it has repeatedly been observed that terminals from the two systems come in close apposition to each other (108-112). The possibilities of a synaptic arrangement between varicosities from adrenergic and cholinergic axons have been studied in more detail in rat iris and heart (113). The pial vessels represent another system in which such close axo-axonal relations regularly occur (1, 107). The partly naked varicosities, identified by potassium permanganate fixation following pretreatment of the animals with 5-hydroxydopamine, approach each other within a fairly wide contact area by 25 nm. This offers a distinct possibility for a functional interaction between the two systems of nerve terminals. No membrane specializations are present to indicate the direction of such functional relationship. It should be noted that close appositions are found also between those varicosities which, at the same time, form contacts with the medial smooth musculature. The above-mentioned studies on the cholinergic modifications of electrically induced noradrenaline release from the perivascular sympathetic nerves in isolated pial vessels have given strong support for the functional nature of this arrangement. Whether also the adrenergic nerves can, in a corresponding way, influence

the neighboring cholinergic nerves remains to be established.

Surprisingly little has been presented on the ultrastructural appearance of the intracerebral vascular innervation. According to Cervós-Navarro and Matakas (23) the axons are located in the adventitial space and are separated from the muscle cells by a distance of at least 80 nm. The terminals are characterized by numerous granular synaptic vesicles varying in size from 25–160 nm. Approximately 66 % of the arterioles studied in the cat's cortex were found to receive innervation. As discussed above a controversial problem concerns the questions whether the intracerebral arterioles are innervated only by adrenergic nerves or whether they also receive cholinergic fibres like the pial vessels (see p. 14), and also whether the adrenergic nerves are only of sympathetic or also of intracerebral origin (see pp. 11–14). Therefore, the electronmicroscopic studies were extended to cats subjected to cranial sympathectomy by removal of the superior cervical ganglia (57). This has previously been found to completely abolish all fluorescent sympathetic nerves in the brain vasculature. In the material so far studied all periarteriolar nerves showed degenerative changes or disappeared at various stages after the operation. This indicates that only sympathetic adrenergic nerves innervate intracerebral arterioles in a conventional manner. This, of course, does not rule out the possibility for transmitter substances and other vasoactive molecules, released from the brain parenchyma, to influence the cerebral microcirculation. Any such influence must then be mediated by other routes than conventional neuro-muscular relations.

Functional aspects

The functional analysis of the relations between the autonomic nerves and the smooth musculature involved in the vasomotor activity of brain vessels has utilized mainly three approaches: (a) transmural electrical stimulation under conditions selectively activating the perivascular nerves without influencing the smooth musculature directly, (b) effects of indirectly acting sympathomimetics such as tyramine, which releases endogenous noradrenaline from the sympathetic nerves, and (c) denervation experiments to elucidate the changes in sensitivity of the receptors to noradrenaline occurring when the sympathetic

nerves as an important transmitter inactivation system are abolished. The studies have thus concentrated on the adrenergic neuro-muscular system, whereas the involvement of the cholinergic nerves has so far been limited to prejunctional phenomena in the neuro-effector area (see pp. 21-22).

(a) Electrical field stimulation was shown to produce a release of tritium activity following preincubation in ^3H -noradrenaline of pial vessels (6), indicating that the adrenergic transmitter is liberated in high amounts from the perivascular nerve terminals. Accordingly, it could be shown with ring segments from the rabbit vertebral-basilar artery system that the perivascular nerves contain sufficient levels of noradrenaline transmitter to produce local vasomotor response of the vessel (114). However, the magnitude of the force developed was usually low which may be due to the fact that, although the vessels contain numerous adrenergic nerves (15) and can take up and accumulate considerable amounts of radioactive noradrenaline (79), a comparatively high proportion of the axons in this particular region is collected in fairly thick bundles of preterminal nature. Furthermore, the complex nature of the neuro-effector system in the pial arteries may allow for conditions where e.g. the cholinergic vasodilation may counteract the sympathetic vasoconstriction during transmural activation of the perivascular nerves. The contractile response to electrical field stimulation has been confirmed for cat's middle cerebral artery (unpublished observations) and human pial arteries (9).

(b) Tyramine is an amine which acts via an uptake into the nerve terminals followed by displacement of the noradrenaline (115, 116). Increasing concentrations of tyramine contracted the pial vessels in vitro (8, 72, 83). This effect could be abolished by sympathectomy or by reserpine treatment and it was also inhibited by blockade of the alpha-receptors with phentolamine (8, 72, 83). Pretreatment with 10^{-6}M cocaine increased the sensitivity of the vascular preparation to tyramine probably due to a combination of a pronounced prejunctional supersensitivity of the receptors to the released noradrenaline and a reduced effectivity of tyramine (117). Urquilla et al. (83), on the other hand, reported a reduced sensitivity of the test system to tyramine with higher concentrations of cocaine. There are reasons to believe that under these conditions the neuronal uptake of tyramine is inhibited by cocaine to an extent

which overcomes the receptor sensitization to noradrenaline. It should also be pointed out that, with the high doses of tyramine that Urquilla et al. used (83), the non-specific direct sympathomimetic action of tyramine may predominate over its specific effect.

(c) Sympathetic denervation produced a 3-fold increase in sensitivity to noradrenaline with regard to its contractile action on cat's middle cerebral artery in vitro (8). A full supersensitivity had developed as soon as the perivascular fluorescent nerves had disappeared. It could be reproduced by cocaine administration (to non-denervated vessels), and it was specific in the sense that the response to acetylcholine was not influenced. This shows that the supersensitivity reaction was of the prejunctional type. The supersensitivity is due to the loss of one important inactivation mechanism for the noradrenaline transmitter in the region of the receptor. Blood vessels have usually shown low prejunctional supersensitivity compared with other smooth muscle tissues (118). A possible explanation for this low sensitization may lie in the relatively larger neuro-muscular distances found in most arteries, including those of the pial system. A correlation between the magnitude of the neuro-muscular interval and noradrenaline potentiation in the presence of cocaine in a variety of sympathetically innervated tissues has therefore been suggested (118). With regard to the neuro-muscular distance found in pial arteries the amount of supersensitivity achieved is in accordance with this correlation. However, in the portal vein, where a neuro-muscular distance of the same order of magnitude has been reported (119), the degree of prejunctional supersensitivity encountered is markedly higher (98a). There is thus reason to believe that structural differences in the detailed construction of the innervation apparatus may be of importance so that a simple relation between neuro-muscular distance and amount of prejunctional supersensitivity does not always exist.

It can be considered well established from available electronmicroscopic and functional studies that the neuro-vascular mechanism in brain vessels is well qualified to act as a mediator for neurogenic control functions in the cerebro-vascular bed.

NEURO-RECEPTOR MECHANISMS IN HUMAN BRAIN VESSELS

Although methods for determinations of local cerebral blood flow in man have reached a high level of technological advancement little, if anything, is yet known about possible neurogenic control mechanisms operating in the human brain circulation. There is again reason to assume that this, to a large extent, is due to the lack of basic knowledge required to design experimental situations simple enough to be applicable to patients but still giving the adequate information needed to make conclusions about complex physiological control functions. It was therefore considered important to find out whether certain anatomical and pharmacological features observed in animal models also hold true for the human cerebro-vascular system. It is difficult to obtain operation specimens of sufficient quantity and quality to perform a detailed analysis, and therefore only some of the aspects studied in the animals were investigated. The human material was obtained from fetuses removed at surgical interruption of pregnancy and from adults during neurosurgical operations.

The observations can be summarized in the following way (9):

(a) Pial as well as intracerebral arteries receive histochemically visible adrenergic nerves, some regions being exceedingly well supplied:

(b) In the pial arterial system there is a similarly dense plexus of acetylcholinesterase-containing nerve fibres.

(c) The two types of autonomic nerves usually run together in the same strands of the plexuses.

(d) The presence of various amine receptors mediating motor effects was established on isolated pial arteries on the basis of reaction and relative potency to certain agonists and the mode of inhibition obtained by specific antagonists. The following receptors were found: contractile alpha-adrenergic, dilatory beta-adrenergic, dilatory cholinergic, and dilatory histamine H_2 -receptors.

(e) The amount of sensitization of pial arteries to noradrenaline induced by cocaine was of a magnitude that would correspond to a neuro-muscular distance of 100 nm.

(f) Transmural stimulation released sufficient amount of adrenergic transmitter from the perivascular nerves to produce a contraction of pial arteries in vitro.

INFLUENCE OF SYMPATHETIC NERVES AND SYMPATHOMIMETIC AMINES ON LOCAL CEREBRAL BLOOD FLOW

The influence of alpha- and beta-receptor stimulants on the cerebral blood flow has for a long time been a matter of great controversy. Direct measurements of cerebral blood flow have thus given a multitude of results. Injection of noradrenaline or adrenaline has been found to decrease the flow (120-125), to have no effects on the flow (126-128), or to induce an increase in the blood flow (129, 130). The beta-receptor agonist, isoprenaline, has given more consistent data. Thus, vasodilation and increase in cerebral blood flow is the most common event (125, 131-133). Direct electrical stimulation of the superior cervical ganglion was early shown to contract pial vessels (for references, see 13, 122) and this has also been confirmed in quantitative measurements of cerebral blood flow (134-139). A recent review on neurogenic control of the cerebral circulation has been presented by Rosenblum (140). The degree of flow reduction has varied greatly in the various experiments, the largest decrease recorded being about 80 % (138). Sectioning of the sympathetic chain in the neck or blockade of its transmission has previously been claimed to have no effect on the cerebral blood flow (see 12, 13). However, new reports (135, 141) have demonstrated increased flow following acute sympathectomy. It was furthermore shown on monkeys that the blood flow on the denervated side of the brain is greater than that on the innervated side (141), whereas autoregulation was equally well demonstrated in both parts of the brain vascular bed.

If we believe that the presence of a rich autonomic innervation of the cerebro-vascular bed indicates the involvement of an autonomic control of the cerebral blood flow, then we have to assume that the inability to define the physiological role of the nerves is due to inadequate knowledge about certain basic neuro-receptor processes in the vascular wall and/or inadequate techniques for recording of certain functional events. During the last few years much information has been gained on the local nervous interaction with the smooth musculature. In order to utilize this information in the design of

adequate functional experiments one would require flow methods which allow for continuous atraumatic measurements also of rapid changes in well-defined regions of non-anaesthetized animals concomitant with recordings of blood gases. The thermoclearance technique (142) possesses these qualities to a considerable extent, and in combination with the ^{14}C -ethanol method (143) it should be possible also to obtain quantitative values of flow changes in restricted regions — particularly when autoradiography is applied — even when the flow is very rapid.

Introductory experiments with the thermoclearance technique have been reported (10, 125, 133, 144); results obtained from the ^{14}C -ethanol method are at present being compiled.

The presently applied thermoclearance method utilizes two probes, one for heating and registration and the other one for measurement of the temperature in the contralateral region. The measuring probe is slightly heated and the time of normalization of the temperature (clearance) is studied and used as an index of flow changes. A negative feedback system assures a constant temperature difference (ΔT) of 0.5°C between the brain region selected for measurement and its corresponding reference area on the contralateral side of the brain. The current (ΔE) necessary to maintain the difference in temperature between the two probes is the parameter recorded. Previous studies have demonstrated a direct linearity between ΔE and blood flow (see 142). It is thus possible to express relative changes in flow, and an estimate of the amount of flow changes can be achieved by comparison with a reference situation. The flow increment produced by inhalation of a given concentration of CO_2 during spontaneous breathing was used as reference. The zero level was determined after cardiac arrest. There is no linearity between blood flow and ΔT which can thus not be used in this type of quantitation. Arterial pCO_2 and pO_2 are measured concomitantly and continuously by mass spectrometry. A thin polyethylene catheter is introduced 2 days before the recordings into the aorta. During the actual recordings in the unanaesthetized animal a metal probe is introduced into the polyethylene catheter and blood gases are "sucked" into the recording system at a vacuum of $10^{-8} - 10^{-9}$ torr.

The caudate nucleus and lateral geniculate body were selected for flow measurements and the thermoprobes were implanted stereotaxically in these

regions on both sides (10). Inhalation of CO_2 in air markedly enhanced the flow in both the caudate nucleus and the lateral geniculate body, concomitant with an increase in arterial pCO_2 . Injection of isoprenaline resulted in a dose-dependent increase in the blood flow of the caudate nucleus, while the effects on the lateral geniculate body were small or negligible. The vasodilation was sometimes even larger than the effect of 7 % CO_2 . The vasodilatory effect of isoprenaline could be prevented by previous injection of the beta-antagonist, propranolol. It was further found (unpublished observations) that the beta₂-receptor agonist terbutaline (145) in a dose of 35 $\mu\text{g}/\text{kg}$ i.v. was without effect on caudate blood flow though, like isoprenaline, it produced a slight fall in blood pressure. The beta₁-receptor antagonist, practolol (0.6 mg/kg i.v.), abolished the effect of 1.3 $\mu\text{g}/\text{kg}$ i.v. of isoprenaline on the blood flow in the caudate nucleus without affecting the slight hypotension. The findings corroborate the in vitro experiments (5) and show that brain vessels possess beta₁-receptors which mediate vasodilation and increased local cerebral blood flow — provided the region is well supplied by sympathetic nerves (see further below).

Systemic injection of noradrenaline or adrenaline caused reduction in the flow of the caudate nucleus, but not in the lateral geniculate body. This decrease could be inhibited by phentolamine, which is a competitive alpha-receptor blocking agent. Similarly, preganglionic stimulation of the sympathetic trunk below the superior cervical ganglion reduced the blood flow, and the effect was less pronounced in the lateral geniculate body than in the caudate nucleus. Also this decrease in flow could be abolished by previous alpha-receptor blockade. The flow was, on the other hand, increased after transection or blockade of the sympathetic trunk in the neck. This indicates that brain vessels are under the influence of a sympathetic tone. The vasoconstrictory action of the sympathetic fibres is mediated by alpha-receptors in agreement with the in vitro studies (5).

Both the beta-agonistic and alpha-agonistic effects were more pronounced in the caudate nucleus; under most of the conditions used the flow in the lateral geniculate body did not show any response at all. This may very well be related

to the fluorescence histochemical finding showing that, although both regions had the same density of arterioles as revealed in tissue sections, 2-3 times more of the arterioles in the caudate nucleus were accompanied by sympathetic adrenergic nerves as compared with the lateral geniculate body.

GENERAL SUMMARY

It has for a long time been a controversial problem whether the cerebro-vascular bed is under nervous influence. When Nielsen and Owman in 1967 (15) demonstrated by fluorescence histochemistry that brain vessels, beyond doubt, are extensively supplied by sympathetic nerve fibres, new possibilities opened up for rational studies on the involvement of neurogenic factors in the control of the cerebral blood flow.

A series of investigations have been reviewed in this thesis in which certain neuro-vascular mechanisms have been investigated by a number of techniques (fluorescence and cholinesterase histochemistry, electron microscopy, in vitro pharmacology including electrical field stimulation, and thermal clearance for cerebral blood flow) applied to a variety of laboratory animals (mouse, rat, hamster, guinea-pig, rabbit, cat) as well as man. The aim of the investigation has been to contribute with information required in the future design of functional experiments to elucidate the physiological role of perivascular nerves in the regulation of blood flow in the brain. The following main results were obtained:

- (a) A well-developed supply of adrenergic nerves, originating in the superior cervical sympathetic ganglia and distributed around extracerebral as well as intracerebral vessels, is a general feature among the various species studied.
- (b) The plexus of perivascular cholinergic nerves is equally well developed but seems to be restricted to brain vessels in the extracerebral system.
- (c) Both the adrenergic and non-adrenergic nerves have been identified in the electron microscope. They occur in approximately equal number and are both related to the outer layer of the medial smooth musculature in a way expected for a true functioning innervation.
- (d) In experiments with tyramine and with transmural electrical stimulation

it could be shown that the perivascular sympathetic nerves can release sufficient amounts of noradrenaline transmitter to produce a local vasoconstriction.

(e) When the perivascular neuronal inactivation mechanism for the adrenergic transmitter is abolished by postganglionic sympathectomy or cocaine treatment the brain vessels develop a 3-fold prejunctional supersensitivity.

(f) The following specific vascular receptors could be identified and further characterized in terms of dissociation constants for the receptor-agonist and/or receptor-antagonist complexes: alpha-adrenergic (mediating contraction), beta₁-adrenergic (dilation), cholinergic muscarinic (dilation in low agonist concentrations, otherwise constriction), and histamine H₂-receptors (dilation).

(g) A close apposition at 25 nm could be demonstrated between varicosities of adrenergic and cholinergic axons in the effector area, indicating axo-axonal interaction. Evidence was obtained to suggest that the cholinergic nerve can act on nicotinic receptors on the neighboring adrenergic nerve to inhibit the release of noradrenaline, and thereby promote vasodilation.

(h) Histochemical and pharmacological in vitro analysis of human brain vessels strongly indicates that the neuro-vascular mechanisms in laboratory animals and man are similar.

(i) In a region of the brain well innervated by sympathetic nerves (caudate nucleus), sympathetic nerve stimulation and exogenous noradrenaline caused marked reduction in blood flow, whereas the flow was enhanced by activation of the beta₁ receptors. In a region which is significantly less innervated (lateral geniculate body) the reactions in the above experiments were small or even absent, suggesting a relation between regional heterogeneity in cerebro-vascular adrenergic innervation and sympathetic influence on local blood flow.

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